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HIF-1 at the Blood-Brain Barrier: A Mediator of Permeability?

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Abstract

Ogunshola, Omolara O and Abraham Al-Ahmad. HIF-1 at the blood-brain barrier: A mediator of permeability? *High Alt Med Biol* 13:153–161, 2012.—The importance of the blood-brain barrier (BBB) in maintaining brain homeostasis cannot be better appreciated than during disease states, where disruption of its function is associated with dramatic detrimental clinical outcome. For decades, neuroscientists and neurobiologists investigated most neurological diseases under the prism of a neuro-centric view, considering the contribution of non-neural components of the CNS (BBB, choroid plexus) negligible or even irrelevant. However, recent reviews have highlighted the importance of BBB breakdown in major neurological diseases.
Hypoxia, as well as hypoxia/reoxygenation, are key components of many neurological diseases and have been shown to contribute to barrier disturbance and dysfunction significantly. Since the master regulator of the hypoxic response, hypoxia inducible factor 1 (HIF-1), is a key determinant for adaptation of cells and tissues to oxygen deprivation, it is likely that this transcription factor also plays a key role in barrier permeability. The possible future use of HIF-1 stabilizers for treatment of diseases characterized by oxygen deprivation to increase neuronal/cell survival means this question is now very pertinent. This review will focus its attention on the role of HIF-1 in BBB breakdown following hypoxic/ischemic injury and the implications for such therapies in a clinical setting.

Key Words: central nervous system, hypoxia inducible factor, microvascular permeability, neurological disease, vascular endothelial growth factor.

The Blood-Brain Barrier

Introduction to the blood-brain barrier: An overview

The central nervous system (CNS) constitutes one of the most important systems present in vertebrates, tightly regulating both vegetative and cognitive functions. In vertebrates, most of the CNS is formed by neurons and glial cells (e.g., astroglia, oligodendrocytes, and microglia). Due to their excitatory nature and their inability to further divide, neurons require a chemically defined and stable extracellular environment, sheltered from any sudden changes in composition. The ability to maintain such a microenvironment is achieved exclusively by the presence of the blood-brain barrier (BBB). The first documented description of the BBB was attributed to Paul Ehrlich (1885) at the end of the 19th century, as he described the absence of chemical dye penetration within the CNS. However, the cellular and molecular nature of the BBB remained unclear for more than 80 years until the publication of two seminal studies by Reese and colleagues. In the first study (Reese and Karnovsky, 1967), the authors described the presence of the barrier at cellular junctions between brain capillary endothelial cells (ECs) lining the cerebral vasculature. Later on, the same authors demonstrated the presence of "tight junction" (TJ) complexes between EC cell junctions that were responsible for the barrier phenotype (Brightman and Reese, 1969).

Although the BBB denomination was originally limited to brain ECs, the current consensus includes it in a multicellular neurovascular unit (Neuwelt et al., 2008; Zlokovic, 2011). The BBB is formed by a monolayer of specialized brain ECs harboring TJ complexes at their cellular junctions. These brain ECs are in direct contact with brain pericytes (Cuevas et al, 1984; Dore-Duffy, 2003), and separated from the brain parenchyma by two layers of extracellular matrices (ECM). The

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inner layer is formed by the vascular basement membrane localized at the EC basolateral side and is shared with neighboring pericytes. The outer layer is formed by the *glia limitans* that surrounds or ensheaths the cerebral vascular tree. Interestingly, in larger vessels these two matrix layers may also be separated by a perivascular space and populated by perivascular cells, particular cell types capable of macrophage activity (Thomas, 1999; Guillemin and Brew, 2004). The main function of this space remains unclear. However, it appears to play an important role in neuroinflammatory diseases (Engelhardt and Coisne, 2011). The glia limitans is formed by polarized astrocyte end-feet processes that directly contact and cover the vast majority of the microvasculature (Senjo et al., 1986). Finally, neurons and microglia contact the brain microvessels, although the specific nature of these additional interactions is also debated.

The blood-brain barrier: The gatekeeper of CNS homeostasis

The BBB plays a crucial role in maintaining CNS homeostasis by acting as a formidable "gatekeeper", regulating the entrance of any chemical and biological entities into the CNS and vice-versa. First, the presence of very "tight" TJ complexes eliminates the passive diffusion of solutes across the endothelium. Second, nonselective transport of small solutes by pinocytosis is practically nonexistent, as brain ECs have a very low pinocytic activity. Therefore, the entrance of most small polar nutrients (glucose, amino acids, etc) through the BBB can only be achieved by the recruitment of specialized solute carriers (Ghersi-Egea et al., 2009). In contrast, lipophilic compounds may reach the brain parenchyma easily via passive diffusion, although the majority of these compounds have only limited bioavailability since the BBB presents an array of various ATP-binding cassettes (ABC) efflux transporters such as P-glycoprotein (P-gp, ABCB1), breast cancer resistance protein (BCRP, ABCG2) and multidrug-resistant associated proteins (MRPs) at their cell surface (Dauchy et al., 2008; Dutheil et al., 2010; Decleves et al., 2011; Hartz and Bauer, 2011). These transporters are highly efficient in removal/efflux of any foreign substances that have managed to penetrate the vascular system from the circulating blood such as drugs and pharmaceutics. Substrates of these transporters have a broad diversity in their chemical structure, making it very difficult to predict whether a newly designed drug candidate may avoid the efflux pump. Indeed current success rates are exceedingly low. In addition, brain ECs are also capable of drug metabolic activity by the presence of certain cytochrome P450 enzymes such including CYP1B1 and CYP2U1 (Dauchy et al., 2008), which may therefore further compromise the diffusion of any spared molecules. The ability of the BBB to metabolize, efflux, or exclude the entrance of foreign substances to the brain efficiently means that it also represents a formidable obstacle for drug entry and treatment of brain pathologies. Taken together, the essential "gatekeeper" function of the BBB is a blessing and a curse for the CNS: it prevents entrance of harmful agents capable of severely compromising brain integrity but also prevents the entrance of pharmaceutical drugs that could restore neuronal function and promote repair mechanisms following injury.

Accumulating experimental evidence supports the hypothesis that opening of the BBB triggers a chain of events leading to neuronal dysfunction and damage resulting in neurological disease (Hawkins and Davis, 2005; Neuwelt et al., 2008; Zlokovic, 2008, 2011; Rosenberg, 2012). When coupled with previous brain insults, additional BBB disruption could have serious detrimental consequences for patient outcome.

Tight junction complexes: The central nervous system great wall

Endothelial cells that line the cerebral capillaries form the anatomic basis of the BBB in higher organisms. Unlike the endothelium of other vascular beds, specialized cerebral microvessel endothelial cells have very low permeability due to the presence of highly organized junctional complexes called tight junctions (TJ). TJ complexes represent highly intimate cell contacts and ensure stringent regulation of CNS homeostasis by severe restriction of the paracellular diffusional pathway between the endothelial cells and substances and/or cells within the circulating blood. These complexes are located within specific TJ strands; brain ECs present three major types of TJ proteins: occludin (Furuse et al, 1993; Hirase et al, 1997), claudins (Morita et al., 1999; Liebner et al., 2000; Krause et al., 2008), and junctional adhesion molecules (JAMs) (Bazzoni 2011). Occludin, claudin, and JAM interactions occur through homotypic interactions (Chung et al., 2001; Nusrat et al., 2005); however recent suggestion of heterotypic occludin interactions additionally imply a certain plasticity and dynamism at the BBB (Nusrat et al., 2005).

Occludin is a 65 kDa tetraspan membrane protein (Furuse et al., 1993) encoded by the *OCLN* gene. Its expression is mostly restricted to epithelial and endothelial cells. Very interestingly, occludin-deficient animals did not present major barrier leakage even at the BBB (Saitou et al., 2000). However, abnormal calcification around cerebral vasculature was noted in these mice, suggesting that occludin may regulate diffusion of bivalent cations such as calcium or magnesium. It is currently suggested that occludin plays a more permeability-regulating role by incorporating itself into the claudin-based strands (reviewed by Förster, 2008). The mechanism by which this occurs, and indeed the precise role(s) of occludin remain to be elucidated. Soon after cloning occludin, the same authors described an additional class of TJ proteins called claudins (Furuse et al., 1998). Claudins are 20–27 kDa tetradomain membrane proteins encoded by 23 different *CLDN* genes in human. Until now, four major claudins have been described at the BBB: claudin-1 (Huber et al., 2001), claudin-3 (Wolburg et al., 2003), claudin-5 (Morita et al., 1999), and claudin-12 (Nitta et al., 2003). Evidence suggests that the claudins constitute the backbone of TJ strands at the BBB (Förster, 2008). Increased expression of claudin-5 in rat brain capillary ECs *in vitro* resulted in decreased monolayer permeability (Ohtsuki et al., 2007). Unlike occludin$^{-/-}$ animals that showed no major vascular leakage, claudin-5$^{-/-}$ mice rapidly died after birth. Although no macroscopic vascular leakage was observed in these animals, Nitta and colleagues (2003) noted an increased permeability to molecules with a molecular weight below 800 Da, suggesting that claudin-5 may infer the highest tightness to the "tight junctions". The effect of deletion of other claudins on the BBB remains undocumented, as these animals rapidly die *in utero* or during the early phase postpartum due to major epithelial lesions. Finally, the third class of protein described at the TJ complexes are represented by the JAMs (Bazzoni, 2011). Unlike occludin

and claudins, JAMs belong to the immunoglobulin (IgG) superfamily and present two extracellular IgG-like domains. ECs express all three different isoforms of JAMs: JAM-A, JAM-B, and JAM-C (Orlova and Chavakis, 2007). JAMs play important roles in modulating barrier function in non-BBB ECs, as well as leukocyte–endothelial interactions. Although the importance of JAMs on BBB function remains largely unclear, decreased JAM-A protein levels following BBB breakdown (Yeung et al., 2008) and an increase in soluble JAM-A following BBB injury (Haarmann et al., 2010) suggests that JAM-A shedding may constitute a biomarker of BBB injury.

Similar to other cell junction proteins, TJ membrane proteins interact with the actin cytoskeleton by soliciting the recruitment of zonula occludens proteins, classically referred to as ZO (Bauer et al., 2010). ZO proteins belong to the membrane-associated guanylate kinase (MAGUKs), as they contain one or several PSD95/Dlg/ZO-1 (PDZ), src-homology3 (SH3), and guanylate kinase (GK) domains. Both ZO-1 and ZO-2 expression at the BBB were reported in the literature (Fischer et al., 2000; Mark and Davis, 2002), whereas proven ZO-3 expression remains undocumented. Interactions between ZO proteins and TJ membrane proteins occur through their PDZ domains, whereas interactions with the cytoskeleton occur through their C-terminus via their actin-binding regions (ABRs). In addition to these distinct domains, ZO proteins present several nuclear localization signals (Bauer et al., 2010), suggesting a certain ability to shuttle between the cytoplasm and nucleus. Thus, ZO proteins may act as transcription factors in addition to their structural scaffold function, although the nature of ZO target genes and their relevance at the BBB remains unknown.

Hypoxia and Blood-Brain Barrier Function

Hypoxia induces blood-brain barrier disruption

Reduction of oxygen levels such that supply fails to meet demand is termed hypoxia. Hypoxia is a strong stimulus for various physiological processes, particularly during development, but is also a major cause or consequence of injury and contributes to progression of many different diseases and pathologies. To ensure survival, hypoxic cells must be able to adapt to oxygen deprivation and switch from aerobic to anaerobic metabolism until oxygen concentrations are restored to manageable levels. Notably, resting oxygen levels, and sensitivity to oxygen deprivation, differ widely in various tissues and organs meaning that hypoxic exposure can have differential effects based on the tissue or cells being studied. The CNS constitutes a system that utilizes an unparalleled degree of physiological resources. With an average blood vessel surface of $10\,m^2$ (Stewart, 1997), it solicits 15%–20% of total cardiac output and 20% of the arterial O_2 input on its own under resting conditions (Kandel et al., 2000). In addition, it heavily relies on glucose as a source of energy by consuming $\sim$20% of daily glucose intake (Zlokovic, 2008). Indeed, such extreme consumption underlies a heavy dependence of the cerebral tissue on constant O_2 and glucose perfusion. Thus, a rapid change in environmental or local O_2 levels may result in dramatic consequences for CNS homeostasis and BBB integrity. Indeed, hypoxia/ischemia may constitute the most frequent cerebrovascular event leading to BBB breakdown.

The effects of hypoxia at the BBB have been extensively investigated. Hypoxia, as well as hypoxia/reperfusion (H/R) stress and cerebral ischemia, have been shown to alter localization and expression of the key junctional proteins ZO-1 and occludin at the BBB in both *in vivo* and *in vitro* BBB models, and correlate with increased paracellular permeability and edema (Abbruscato and Davis, 1999; Mark and Davis, 2002; Witt et al., 2003; Kago et al., 2006; McCaffrey et al., 2009). The effect of hypoxia on BBB function was also demonstrated to reduce claudin-5 expression levels and increase paracellular permeability of low molecular weight compounds after exposure of brain ECs and retinal flatmounts to hypoxia (Koto et al., 2007). The disruption of the BBB in hypoxic conditions is multi-factorial and may involve factors such as enhanced production of vascular endothelial growth factor (VEGF), nitric oxide (NO), and inflammatory cytokines (reviewed by Ballabh et al., 2004; Kaur and Ling, 2008). Increased cytokines and subsequent upregulation of endothelial and neutrophil adhesion molecules lead to leucocyte adhesion and transmigration across the endothelium and the BBB creating a positive feedback loop that further enhances vascular damage (Ballabh et al., 2004).

Notably, ischemia or hypoxia-induced alterations in BBB TJs have not been observed in all studies. This may be related to differences in the severity of hypoxia within different areas of the brain following insult, the differential cell-response of surrounding cell types, as well as the duration of the injury (Al Ahmad et al., 2009). Understanding the inter-related and complex contributions of these parameters to the modulation of barrier function may be of significant relevance in the design of future therapeutics.

Hypoxia-mediated hypoxia inducible faxtor-1 signaling

Hypoxia induces a variety of signaling pathways. The most widely studied mediators of the hypoxic response are the family of transcription factors known as hypoxia inducible factors (HIFs). There are 3 known members of the HIF family, namely HIF-1, 2, and 3, with HIF-1 being the most well characterized and generally considered the master regulator of the hypoxic response. HIF-1 mediates many adaptive endogenous mechanisms during hypoxic brain injury by transcriptional activation of specific target genes that function to restore oxygen supply.

HIFs are heterodimeric transcription factors consisting of an oxygen-inducible α subunit (HIFα) and an oxygen-independent subunit (HIFβ, also known as Aryl Hydrocarbon Nuclear Translocator; ARNT) (Wang and Semenza, 1993; Ratcliffe et al., 1998; Semenza, 2002). These subunits are differentially localized, with HIFα being expressed in the cytoplasm whereas ARNT is a nuclear protein. Under normal conditions (i.e., in the presence of oxygen), the HIFα protein is constantly degraded due to hydroxylation of specific proline residues by enzymes called prolyl hydroxylases (PHDs) (Ivan et al., 2001; Jaakkola et al., 2001; Semenza, 2001; Maxwell and Ratcliffe, 2002). This modification leads to recognition by the Von Hippel Lindau protein, ubiquitination, and degradation by the E3 ligase machinery. When oxygen is reduced and becomes limiting, the PHD enzymes are inhibited. As a result, HIFα is no longer degraded but accumulates and after phosphorylation is transported to the nucleus where it binds ARNT, forming the functional HIF protein. Thereafter, the heterodimer forms a complex with a number of target proteins by binding the hypoxic response element (HRE) in their promoter regions and subsequently induces their expression. To date, a large number of HIF target genes have been

identified, many being involved in the switch from aerobic metabolism to glycolysis, as well as increased angiogenesis and erythrocytosis (i.e., changes that reduce energy consumption and promote re-establishment of oxygen delivery), thus facilitating cellular adaptation to oxygen deprivation, as well as elevating a number of target genes involved in tissue repair (Hofer et al., 2002; Maxwell and Ratcliffe, 2002; Hopfl et al., 2004).

Thus, HIF-1 is largely considered to be essential for cellular survival during injury and has been reported to protect neurons from apoptosis caused by oxidative stress and focal cerebral ischemia (Digicaylioglu and Lipton, 2001; Grimm et al., 2005; Liu et al., 2005). Stimulation of HIF-1α upon hypoxic preconditioning or chemical induction of HIF-1α was also shown to induce HIF-1 target pro-survival genes such as VEGF and EPO, resulting in increased cell survival (Digicaylioglu and Lipton, 2001; Bereron et al., 2000; Stenzel-Poore et al., 2003) and neuroprotective effects (reviewed by Ferriero, 2005). HIF-1-induced angiogenesis and glycolysis also increased delivery of oxygen and nutrients that are critical for cell survival under hypoxic/ischemic conditions (Bergeron et al., 2000). Furthermore, neuron-specific knockdown of HIF-1α was demonstrated to increase tissue damage and reduce survival of mice subjected to middle cerebral artery occlusion (Baranova et al., 2007).

Although HIFs are essential for cellular adaptation to reduced oxygenation, over the last few years it has become apparent that these transcription factors can act as double-edged swords. Indeed, a wealth of contrasting data suggests that, in addition to cellular adaptation and survival, HIFs also contribute to activation of cellular processes that lead to apoptosis and necrosis. Several groups have reported detrimental effects of HIF-1 in cerebral ischemia. For instance, Halterman et al. (1999) reported that HIF-1α coordinated the activity of p53 in driving ischemia-induced delayed neuronal death instead of providing neuroprotection. HIF-1 was shown *in vitro* to mediate hypoxia-induced growth arrest and apoptosis (Goda et al., 2003) and regulate the expression of proapoptotic family members such as Bcl-2 adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and caspase-3 that are increased following cerebral ischemia (Guo et al., 2001; Schmidt-Kastner et al., 2004; Althaus et al., 2006; Van Hoecke et al., 2007). Furthermore, brain-specific knockdown of HIF-1α reduced ischemic damage in knockout mice and was neuroprotective (Helton et al., 2005).

Overall, current data suggests that mild or acute hypoxia induces adaptive gene expression such as EPO, Glut1, and VEGF, whereas severe or sustained hypoxia HIF-1α can lead to activation of prodeath genes, such as BNIP3, COX2, or p53 stabilization (reviewed by Chen et al., 2009). Thus, HIF-1 can activate transcription factors and signaling pathways with both pro-death and pro-survival functions. The outcome of HIF-1 induction appears to be dependent on the duration (Halterman and Federoff, 1999), the pathological stimuli, and the cell type in which it is induced (Vangeison et al., 2008).

Thus, it is apparent that during oxygen deprivation, cell-specific temporal-spatial modulation of crucial HIF-1 signaling pathways is a key determinant of functional outcome. It is also essential to keep in mind that activation of the pathway under certain circumstances may also have negative outcomes, especially if the system is chronically activated/induced.

Besides regulation by hypoxia, other signaling pathways can also modulate HIF activation. An example is evidence for a role of mitochondrial ROS in cellular oxygen sensing in at least some cell types (Harten et al., 2010). The contribution of oxygen sensing, redox status, and a variety of molecular factors and pharmacological agents to HIF stabilization has recently been reviewed by Chen et al. (2009) and Martinez-Sanchez and Giuliani (2007).

Hypoxia Inducible Factor-1 Signaling at the Blood-Brain Barrier: From Pathophysiology to Therapeutic Perspectives

Effect of hypoxia inducible factor-1 on barrier permeability

As stated above, it is known that hypoxia compromises barrier integrity; however, the precise mechanisms that mediate barrier dysfunction remain largely unknown. The role of HIFs and their target genes in major cellular alterations and adaptations in response to oxygen deprivation suggests they may be instrumental modulators of BBB integrity. Indeed, current data from our group and others suggest that HIF-1 is a likely mediator of barrier disruption. A possible role for either HIF-2 or 3 at the BBB is yet to be addressed.

A study by Witt et al. (2005) first suggested that transcription factors such as HIF-1 and NFκB are upstream mediators of TJ protein alterations during hypoxia and H/R, which may involve VEGF induction and expression. Indeed, VEGF is a strong inducer of vascular permeability, and increased VEGF levels positively correlate with changes in TJ redistribution of ZO-1 and occludin, as well as with alterations in the actin cytoskeleton both *in vivo* and *in vitro* (Antonetti et al, 1998; Liu et al., 2001; Pedram et al., 2002). Yeh et al. (2007) subsequently demonstrated that 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1), an inhibitor of HIF-1α, was able to prevent increased permeability in adult rat brain ECs in response to chemical hypoxia, likely through inhibition of HIF-1α accumulation and VEGF production. Pretreatment with YC-1 also seemed to reduce ischemia/reperfusion-induced increase of BBB permeability and HIF-1α accumulation in a rat *in vivo* model (Yeh et al., 2007). Elevation of HIF-1α was shown to be harmful in cerebral ischemia after 2 h of MCA occlusion, and inhibition of HIF-1α and VEGF by 2ME2 and D609 could protect against brain damage by reducing neuronal expression of BNIP3, and cleaved caspase 3 during stroke (Chen et al., 2009). Acute inhibition of HIF-1α (when administered within a 3 hour window) was also neuroprotective in neonatal hypoxic-ischemic injury by preserving BBB integrity and reducing brain edema (Chen et al., 2008). Interestingly however, most of the *in vivo* studies have not demonstrated convincingly that the crucial HIF modulation takes place in the ECs, indeed in most cases, the neurons were the most sensitive and robustly upregulated HIF-1.

Very recently, it was demonstrated that, despite ameliorating ischemia-induced BBB disruption (determined by Evans blue leakage), YC-1 did not alter brain edema formation and significantly exaggerated ischemic brain damages in terms of infarct volume and mortality as evaluated by MRI and histological staining (Yan et al., 2011). The data indicate that BBB protection resulting from HIF-1 inhibition by YC-1 contributes little to the overall brain tissue injury induced by cerebral ischemia—a finding that needs to be further investigated. However, the study clearly implies that the presence of HIF-1 is critical in promoting neuronal survival during ischemia/reperfusion and HIF-1 modulation may have differential effects on ischemic outcome and BBB permeability.

Notably, detrimental effects of HIF-1 on BBB function may not only be limited to stroke-related events. In an *in vivo* model of traumatic brain injury (TBI), brain edema was significantly decreased after inhibition of HIF-1α (Higashida et al., 2011). HIF-1α activation in the brain of dystrophic mouse (model for Duchenne muscular dystrophy) was also suggested to be partly responsible for both BBB opening and increased angiogenesis through reduced protein levels and increased phosphorylation of ZO-1 as well as an upregulation of VEGF and VEGFR-2 expression (Nico et al., 2007). HIF-1α was shown to be involved in brain edema formation and BBB disruption via a molecular signaling pathway involving aquaporin-4 and matrix metalloproteinase-9 in a subarachnoid hemorrhage model (Wang et al., 2012). Other data strongly indicate that HIF-1 plays an important role in high glucose-induced BBB dysfunction (Yan et al., 20120). Upregulating HIF-1 activity by cobalt chloride increased the paracellular permeability of ECs exposed to normal glucose, whereas downregulating HIF-1 activity, by HIF-1α inhibitors and HIF-1α specific siRNA, ameliorated the redistribution of occludin and ZO-1 and increased permeability induced by high glucose (Yan et al., 2012). The involvement of HIF-1 in impaired junction assembly in the kidney has also been reported (Harten et al., 2010). The precise mechanisms by which HIF-1 modulates TJ proteins are still to be fully elucidated. Hypoxia also rapidly stimulates cytoskeletal reorganization *in vitro* (Park et al., 1999; Brown and Davis, 2005) and *in vivo* (Kaur and Ling, 2008; Bauer et al., 2010), and attachment of TJ proteins to the actin cytoskeleton via accessory proteins (Vorbrodt et al., 2003) means that barrier permeability is also closely linked to such changes. Cytoskeletal rearrangements also result in cellular movement, such as endothelial migration leading to angiogenesis and retraction of astrocyte endfeet from vascular walls, both of which contribute to barrier breakdown. Whether these effects are HIF-1 dependent or independent is still to be addressed.

Cell-specificity of hypoxia inducible factor-1 mediated responses and their effect on blood-brain barrier integrity

Although current concepts advocate that conserved hypoxic adaptive mechanisms occur in many different cell types of the brain, specific temporal-spatial modulation of crucial pathways such as HIF signaling may be the key determinant of functional outcome. Thus the impact of activation of the HIF-1 pathway on the response of individual BBB cells and subsequent barrier function is a pertinent issue that needs to be tackled.

Our studies clearly indicate that individual responses of barrier cells to O_2 deprivation define the outcome of hypoxic injury and the integrity of the BBB (Al Ahmad et al., 2009; 2010). The contribution of HIF-1 induction by barrier modulating cells, astrocytes and pericytes as well as neurons themselves, to barrier stability is still a largely open question. Unlike many other cells, astrocytes are very resistant to hypoxic stress. We showed that severe oxygen deprivation is required to induce HIF signaling and modulate subsequent survival and proliferation in astrocytes (Schmid-Brunclik et al., 2008). However, our data also indicate that VEGF induction during hypoxia in these cells likely occurs through HIF-1 dependent and independent mechanisms. Our current investigations advocate that pericytes are also hypoxia-resistant cells (Engelhardt and Ogunshola, unpublished data) although fully comparable studies are still to be completed. Notably, astrocytes and pericytes have differential responses to hypoxia that are important for barrier regulation depending on the duration and severity of insult (Al Ahmad et al., 2009). Although impairment and/or alteration of either astrocyte or pericyte function results in microvascular damage and accelerates neuronal death (Ballabh et al., 2004; Persidsky et al., 2006; Wolburg-Buchholz et al., 2008), the contribution of activation of HIF-1 signaling in these cells to BBB leakage and central nervous system edema formation remains largely unknown. Interestingly, it was recently demonstrated that, although loss of HIF-1α function in neurons reduced neuronal viability during hypoxia, selective loss of HIF-1 function in astrocytes markedly protected neurons from hypoxic-induced neuronal death (Vangeison et al., 2008). Notably, a systematic *in vivo* and *in vitro* investigation of cell-specific responses mediated via HIF-1 is warranted to provide detailed knowledge of physiological and pathological barrier modulation, and define future targets for development of rational therapeutic approaches that optimize recovery.

Hypoxia inducible factor-1 stabilization as a therapeutic target

Therapeutic activation of HIF-1 is likely to mimic, at least in part, the effects of hypoxia preconditioning. Indeed, it has been suggested that certain protective agents used to treat stroke may act via HIF induction (reviewed by Harten et al., 2010). However, a major caveat, clear from the studies outlined above, is that not all consequences of HIF activation may be beneficial and some could even be deleterious. In general, the therapeutic potential of development and use of small molecule HIF stabilizers (PHD inhibitors) to improve cell survival after injury is gaining popularity in many different fields. Such drugs could provide significant protection for a variety of different cells during injury and pathological situations such as stroke and TBI. Since the first indications are that HIF-1 may disturb barrier function, the applications of such therapeutics must be treated with caution. Enhanced edema, and influx of other blood-borne molecules as a result of increased BBB permeability, will increase intra-cranial pressure and the transport of potentially detrimental substances into the brain parenchyma. Additionally, the doses applied and the chemical toxicity of the compounds could also significantly modify cellular responses and contribute to damage. Thus the adverse effects of reduced barrier integrity must be carefully assessed when administering HIF-1 stabilizing drugs. It must also be emphasized, however, that, as suggested by *in vitro* and *in vivo* studies, the duration of the stabilization of HIF-1 and the regions being targeted will likely be instrumental in obtaining a positive outcome during and after treatment and must also be taken into consideration. A number of reviews on the effects and use of PHD inhibitors and HIF-1 stabilizers as therapeutics have recently been published (Han et al., 2010; Harten et al., 2010; Miyata et al., 2011).

On the other hand, one could propose that such drugs be used to induce a partial opening of the barrier and thus facilitate drug entry into the brain parenchyma. Such a strategy using combination therapies could have high benefit under particular scenarios. Indeed, selective opening of the

endothelium tight junctions to facilitate drug delivery to the brain is also an area of intense research, since delivery of therapeutics to the CNS remains highly challenging (Ogunshola, 2011; Rajadhyaksha et al., 2011). Of course, the feasibility of such an approach, although very attractive, remains to be properly researched to ensure exploitation of the benefits of BBB modulation, while minimizing the potential damage.

Conclusion

Maintenance of BBB integrity and thus brain homeostasis is crucial for brain cells, and highly sensitive neurons in particular, to be able to function. During hypoxia/ischemia, the BBB is compromised, resulting in alterations that can have significant detrimental effects. Thus, identification of the mediators of barrier dysfunction may provide not only important avenues to prevent barrier permeability but also perhaps ways to selectively modulate barrier function and facilitate the passage of protective drugs and therapeutics. Current data suggest that HIF-1 may represent such a mediator; but more knowledge of the regulation of specific barrier properties during different injury paradigms and windows of opportunity is required. Better assessment of the positive versus the negative effects of acute versus chronic stabilization of HIF-1 is also needed. Overall considering its multifunctional role, differential effect on different cells and double-edged sword mode of action, it seems unlikely that HIF-1 stabilization on its own will be the magic solution to improving brain cell survival after injury—but perhaps combination therapies will provide significant gains. Currently many questions remain unanswered, but given that HIF-1 regulates the transcription of numerous genes, a more global approach to its pleiotropic action at the BBB will undoubtedly provide better clarification of its therapeutic potential.

Author Disclosure Statement

No competing financial interests exist.

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